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## **Cilengitide: an RGD pentapeptide $\alpha_3$ and $\alpha_5$ integrin inhibitor in development for glioblastoma and other malignancies**

Reardon, D A ; Neyns, B ; Weller, M ; Tonn, J C ; Nabors, L B ; Stupp, R

**Abstract:** Cilengitide, a cyclicized arginine-glycine-aspartic acid-containing pentapeptide, potently blocks  $\alpha_3$  and  $\alpha_5$  integrin activation. Integrins are upregulated in many malignancies and mediate a wide variety of tumor-stroma interactions. Cilengitide and other integrin-targeting therapeutics have preclinical activity against many cancer subtypes including glioblastoma (GBM), the most common and deadliest CNS tumor. Cilengitide is active against orthotopic GBM xenografts and can augment radiotherapy and chemotherapy in these models. In Phase I and II GBM trials, cilengitide and the combination of cilengitide with standard temozolomide and radiation demonstrate consistent antitumor activity and a favorable safety profile. Cilengitide is currently under evaluation in a pivotal, randomized Phase III study (Cilengitide in Combination With Temozolomide and Radiotherapy in Newly Diagnosed Glioblastoma Phase III Randomized Clinical Trial [CENTRIC]) for newly diagnosed GBM. In addition, randomized controlled Phase II studies with cilengitide are ongoing for non-small-cell lung cancer and squamous cell carcinoma of the head and neck. Cilengitide is the first integrin inhibitor in clinical Phase III development for oncology.

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**Cilengitide: An RGD-Pentapeptide  $\alpha v\beta 3$  and  $\alpha v\beta 5$  Integrin Inhibitor in Development for  
Glioblastoma and other Malignancies**

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Running title: Cilengitide for glioblastoma

Keywords: angiogenesis, glioblastoma, integrins, malignant glioma, vascular endothelial growth factor

Abbreviations List: AA, anaplastic astrocytoma; AO, anaplastic oligodendroglioma; AOA, anaplastic oligoastrocytoma; AST, aspartate aminotransferase; CNS, central nervous system; CR, complete response; DLT, dose-limiting toxicity; EIAEDs, enzyme-inducing antiepileptic drugs; GBM, glioblastoma multiforme; ITT, intent-to treat; KPS, Karnofsky performance status; MG, malignant glioma; MR, marginal response; MTD, maximum-tolerated dose; New Agents Brain Tumor Treatment Consortium (NABTT); North American Brain Tumor Consortium (NABTC); OS, overall survival; PD, progressive disease; PFS, progression-free survival; PR, partial response; SD, stable disease; TMZ, temozolomide; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor; XRT, external beam radiotherapy

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## Summary

Cilengitide, a cyclicized RGD-containing pentapeptide, potently blocks  $\alpha v\beta 3$  and  $\alpha v\beta 5$  integrin activation. Integrins are upregulated in many malignancies and mediate a wide variety of tumor-stroma interactions. Cilengitide and other integrin-targeting therapeutics have preclinical activity against many cancer subtypes including glioblastoma (GBM), the most common and deadliest central nervous system (CNS) tumor. Cilengitide is active against orthotopic GBM xenografts and can augment radiotherapy and chemotherapy in these models. In phase I and II GBM trials, cilengitide and the combination of cilengitide with standard temozolomide (TMZ) and radiation demonstrate consistent anti-tumor activity and a favorable safety profile. Cilengitide is currently under evaluation in a pivotal, randomized phase III study (CENTRIC) for newly diagnosed GBM. In addition, randomized, controlled phase II studies with cilengitide are ongoing for non-small cell lung cancer and squamous cell carcinoma of the head and neck. Cilengitide is the first integrin-inhibitor in clinical phase III development for oncology.

## Introduction

The annual incidence of GBM in the United States is approximately 3.15 cases per 100,000 [1]. Extrapolation of this rate to the current global population (6.8 billion) projects that more than 210,000 GBM patients will be diagnosed worldwide each year. Despite aggressive, multimodality therapy, outcome for GBM patients remains dismal. Specifically, the current standard of care, including maximum safe resection followed by radiation and temozolomide chemotherapy (XRT/TMZ), is associated with a median overall survival (OS) of 14.6 months and a 5-year survival rate of only 10% [2, 3]. Recurrence remains nearly inevitable and salvage therapies have historically achieved limited benefit [4-6]. However, in May, 2009, the USA Food and Drug Administration granted accelerated approval to bevacizumab, a humanized monoclonal antibody targeting vascular endothelial growth factor (VEGF), for recurrent GBM based on durable radiographic responses [7, 8]. Of note, bevacizumab achieved an OS of 8-10 months in these studies, which is similar to the results of single agent cilengitide in the recurrent

setting [9]. Nonetheless, more effective therapies for newly diagnosed as well as recurrent GBM patients remain desperately needed.

Integrins, a 24 member family of heterodimeric transmembrane receptors composed of paired alpha and beta subunits, regulate cell-to-cell and cell-stroma interactions. Based on their respective alpha and beta chain pairings, integrins bind specifically to key components of the extracellular matrix, including vitronectin, fibronectin, laminin, fibroblast growth factor, matrix-metalloproteinase (MMP)-2, thrombospondin, osteopontin, collagen, fibrin and fibrinogen. Integrin binding can be redundant in that more than one integrin can bind to the same ligand, as well as promiscuous, in that most integrins can bind multiple ligands. Although integrins lack intrinsic kinase activity, ligand binding activates integrins to form focal adhesion complexes in the cell membrane, composed of clusters of integrins with signaling and adapter proteins [10]. These complexes recruit focal adhesion kinase which autophosphorylates and then activates downstream intracellular signaling pathways including the NF- $\kappa$ B [11, 12], PI3/Akt[13], SRC[14] and ras/MAPK kinase [15, 16] cascades.

Integrins, especially alpha v integrins, are attractive therapeutic targets for malignant glioma [17] and other malignancies because they regulate a diverse array of critical tumor behaviors including cell signaling, survival proliferation, invasion and angiogenesis[18]. Integrins play a particularly important role in tumor cell invasion and migration [19-22]. Activated integrins in focal adhesion complexes regulate remodeling of the intracellular actin cytoskeleton which directs cytoplasmic flow, while ligand binding by the extracellular component of integrins provides traction to direct cell movement [10]. The activation of integrins by ligand binding also generates critical intermediaries in several aspects of the host tumor response including angiogenesis, pericyte recruitment with stabilization of tumor vasculature, as well as the infiltration and activation of myeloid cells, bone marrow derived precursors, fibroblasts and platelets into the tumor microenvironment [18, 23-26]. Integrins have also recently been implicated in several aspects of tumor stem cell biology. Specifically, integrin expression characterizes some stem cell

populations [27, 28], and integrins can regulate the maintenance of stem cell subpopulations [29] as well as the expression of some stem cell markers [30]. In particular, the  $\alpha 6$  integrin is an important regulator of GBM stem cells [31]. Another recently identified aspect of GBM biology involving integrins is the regulation of HIF-1 $\alpha$  and tumor hypoxia, a critical regulator of tumor therapeutic response [32]. Based on the numerous and diverse repertoire of integrin activities in cancer biology, agents that can selectively block integrin activity offer multiple mechanisms of potential anti-tumor activity. Furthermore, integrin targeting may also potentially enhance the actions of many types of cancer therapeutics including radiation therapy, cytotoxic chemotherapy, cell signaling inhibitors, immunotherapeutics, vascular targeting agents and anti-angiogenics.

Cilengitide potently and selectively blocks activation of the  $\alpha v\beta 3$  and  $\alpha v\beta 5$  integrins, which are upregulated in several cancers including GBM [33-35]. Preclinical studies demonstrate that cilengitide monotherapy has anti-tumor activity in xenograft tumor models and cilengitide can augment the activity of radiation and chemotherapy, including TMZ [36-40]. Cilengitide monotherapy and the combination of cilengitide with standard temozolomide (TMZ) and radiation have demonstrated consistent anti-tumor activity as well as a highly favorable safety profile across a spectrum of phase I and II clinical trials for both recurrent and newly diagnosed GBM patients [9, 41-44]. Cilengitide is currently being evaluated in a multi-national, randomized, pivotal phase III study (CENTRIC) for newly diagnosed GBM patients with methylation of the methylguanine-methyltransferase (MGMT) gene promoter.

## **Overview of the market**

Effective therapy for patients with GBM, the most common, primary malignant CNS tumor among adults, remains a major challenge in oncology. Standard treatment for newly diagnosed patients includes maximum safe resection when feasible followed by daily involved field external beam radiotherapy to approximately 60 Gy with concomitant TMZ chemotherapy, and then 6 months of adjuvant TMZ. In a pivotal randomized trial, this therapeutic approach led to a median OS of 14.6 months and a 5-year

survival rate of 10% [2, 3]. Some of the DNA damage induced by TMZ is repaired by MGMT, a ubiquitous DNA repair protein [45]. Approximately 40% of tumors from newly diagnosed GBM patients demonstrate methylation of the MGMT gene promoter, leading to relative silencing of gene expression and protein production [46]. Retrospective analysis of MGMT methylation status demonstrated that patients whose tumor have an unmethylated MGMT promoter derive minimal benefit from the addition of TMZ to radiotherapy [47]. Prospective evaluation of the impact of MGMT methylation is pending analysis of a recently completed phase III study for newly diagnosed GBM patients (RTOG 0525/EORTC 26052 Intergroup Study). At recurrence, treatment options are limited and offer modest benefit and no treatment has demonstrated significantly improved overall survival in a randomized controlled trial. Recent meta-analyses of salvage regimens demonstrate rates of overall radiographic response and progression-free survival at six months of only 5-10% [4, 5]. Therefore there remains a critical need to identify novel agents with anti-tumor activity for both newly diagnosed and recurrent GBM patients.

Several factors likely undermine the efficacy of therapeutic agents directly targeting GBM tumors, including molecular genetic heterogeneity across and within tumors, complex and redundant activation of intracellular signaling pathways regulating proliferation, invasion, angiogenesis and survival, genetic instability leading to high rates of de novo and acquired resistance mechanisms, and restricted delivery of pharmacologic agents into the CNS and tumor microenvironment due to the blood-brain-barrier and high interstitial fluid pressures within the tumor mass [48]. In contrast, targeting factors in the tumor microenvironment may prove less susceptible to these limitations. Targeting vascular endothelial growth factor (VEGF) and integrins are prototypic examples of factors that play key roles in the tumor microenvironment and are the focus of much recent clinical investigation.

GBM are highly vascularized tumors and VEGF is a leading regulator of GBM angiogenesis [49-54]. VEGF-directed therapies exert a marked anti-tumor effect in orthotopic, GBM xenograft models [55, 56]. Initial clinical studies confirmed that the humanized anti-VEGF monoclonal antibody bevacizumab could

be safely administered to brain tumor patients and was associated with encouraging rates of clinical and radiographic improvement in recurrent GBM patients [57, 58]. Two follow-up studies that incorporated stringent, independent radiographic review confirmed these findings and were the basis of accelerated approval of bevacizumab by the US Food and Drug Administration (FDA) for recurrent GBM in 2009. These studies included a single-arm phase II trial of bevacizumab monotherapy for heavily pre-treated, recurrent GBM patients [8] and a multi-center, phase II study that randomized GBM patients at first or second recurrence to either bevacizumab monotherapy or bevacizumab plus irinotecan [7]. The rates of radiographic response and progression-free survival at six months (PFS-6) in these studies were approximately 28-35% and 29-43%, respectively. Of note, the European Medicinal Agency voted to deny approval based on these data primarily due to the lack of a non-bevacizumab control arm[59]. However, randomized, controlled clinical trials with bevacizumab are planned to be initiated by the EORTC in 2011 for patients with recurrent GBM and recurrent grade II-III gliomas, respectively.

Angiogenesis has also been targeted in GBM using a variety of VEGF receptor (VEGFR2) tyrosine kinase inhibitors (TKIs), most of which also inhibit other relevant receptor TKIs including PDGFR, FGF, c-met and c-KIT. Cediranib, a TKI targeting VEGFR2, c-KIT and PDGFR, demonstrated evidence of anti-tumor benefit in a multi-center phase II study when administered as a single agent [60]. However, a pivotal, randomized phase III study of cediranib alone or in combination with lomustine (CCNU), recently reported that single agent cediranib or cediranib with lomustine (CCNU) failed to prolong PFS compared to CCNU alone [61]. Pazopanib and sunitinib, two additional multi-targeted VEGFR TKIs, were recently demonstrated to have limited single agent activity among recurrent malignant glioma patients[62] [63]. Evaluation of VEGF/VEGFR inhibitors is also underway for newly diagnosed GBM patients including two, randomized, multi-national, placebo-controlled, phase III studies randomizing patients to either standard XRT/TMZ plus placebo or XRT/TMZ plus bevacizumab.



Several additional agents are also being evaluated in ongoing clinical trials for newly diagnosed GBM patients. A phase III study evaluating CDX-110, a peptide vaccine targeting the epidermal growth factor receptor vIII mutant (EGFRvIII) was initiated based on supportive preclinical data [64, 65], as well as encouraging overall survival achieved in a single-arm study of newly diagnosed GBM patients [66]. However the study discontinued prematurely due to a high rate of drop out among patients who randomized to standard therapy without the vaccine. A follow-up phase III study that includes a blinded placebo control group is under consideration. This study will only include patients with tumors that express EGFRvIII, which typically includes 30-40% of the GBM population [67, 68]. Finally, a phase III study was recently initiated to evaluate the use of an electromagnetic field generating device (Novocure) based on modest anti-tumor benefit observed among heavily pre-treated recurrent GBM patients [69].

Integrins, and especially integrins  $\alpha\beta3$  and  $\alpha\beta5$ , are widely expressed by many varied cell types in the tumor microenvironment including endothelial cells, pericytes, infiltrating myeloid cells, monocytes, bone marrow derived precursor cells and fibroblasts [18]. In addition, many tumors directly express integrins. For example, GBMs frequently express  $\alpha\beta3$  and  $\alpha\beta5$  integrins [33, 35, 70, 71]. Furthermore, multiple integrin ligands are abundantly expressed in the GBM microenvironment [72-74]. Integrin activation via ligand binding is associated with several critical aspects of tumor biology, including growth factor signaling, proliferation, survival, invasion, angiogenesis, and the host tumor response [18]. Thus, blocking integrins may lead to multifaceted mechanisms of anti-tumor activity.

There are four classes of integrin targeting therapeutics currently in clinical evaluation including monoclonal antibodies, peptidomimetic and non-peptidomimetic molecules as well as miscellaneous (Table 1). Monoclonal antibodies (MAbs) directed against the extracellular domain can effectively block the ligand binding site of integrins. Several integrin-targeting MAbs are in advanced clinical evaluation including the following: etaracizumab (MEDI-522; Abegrin; MedImmune), a MAb that specifically targets  $\alpha\beta3$ ; intetumumab (CNTO 95; Centocor, Inc. Malvern, PA), a humanized MAb targeting the  $\alpha\beta5$

extracellular domain; DI-17E6 (EMDSerono, MerckKGaA, Darmstadt, Germany), a de-immunized Mab that targets the  $\alpha_v$  extracellular domain; and volocixumab (M200; PDL BioPharma, Incline Village, NV, USA and Biogen Idec, Weston, MA, USA), a chimeric MAb that targets  $\alpha_5\beta_1$ , an integrin particularly associated with tumor angiogenesis [75]. Of note, phase I studies in advanced solid tumor patients with each of these MABs confirmed their overall safety and lack of “class-associated” toxicities; in fact, a maximal tolerated dose (MTD) could not be identified in any of these dose-escalation trials. In addition, evidence of anti-tumor activity was observed despite the dose escalation study design [76-78]. Only one phase II study has been reported with these agents to date, [79] but several phase I/II studies are ongoing in many solid tumor types including melanoma as well as colorectal, prostate, pancreatic and ovarian cancers.

A second class of integrin inhibiting therapeutics is the small peptidomimetic molecules that include short peptide sequences to specifically block ligand/integrin binding sites. Several examples of this class compete for the arginine-glycine-aspartic acid (RGD) peptide sequence that regulates binding of ligands such as vimentin and fibronectin to specific integrins including the  $\alpha_v$ -containing integrins and  $\alpha_5\beta_1$ . Of note, cyclization of the RGD binding inhibitors enhances stabilization by 10-100 fold compared to the open ring peptides [80]. Several of these inhibitors are in early clinical development. Of note, 18F-labeled glycosylated Arg-Gly-Asp peptide ([18F]Galacto-RGD) positron emission tomography (PET) has been demonstrated to be feasible in recurrent GBM patients and tracer uptake correlated with immunohistochemical  $\alpha_v\beta_3$  integrin expression of corresponding tumor samples [81].

Non-peptidomimetics represent the third class of integrin inhibitors, many of which are orally bioavailable due to lack of peptide bonds. Several of these agents are in preclinical and early clinical evaluation. In addition, a few miscellaneous agents that can suppress integrin activity are in clinical development.

## Introduction to the compound

Cilengitide, (EMD 121974), a cyclized RGD-containing pentapeptide that selectively and potently blocks activation of the  $\alpha v\beta 3$  and  $\alpha v\beta 5$  integrins [82], is manufactured by Merck KGaA, Darmstadt, Germany.

## Chemistry

Cilengitide is the inner salt of the cyclized pentapeptide c-[Arg-Gly-Asp-DPhe-(NMeVal)]. Its chemical formula is  $C_{27}H_{40}N_8O_7$  (Figure 1). Cilengitide has a molecular weight of 588.7 atomic mass units.

Cilengitide is formulated as a sterile aqueous solution provided in glass vials for intravenous administration.

## Pharmacokinetics and metabolism

The primary pathway for cilengitide excretion is renal. Using  $^{14}C$ -labeled cilengitide in Cynomolgus monkeys, approximately 90% of the injected radioactivity was detected in the urine as unchanged drug in less than 72 hours.

Results from a number of clinical trials reveal consistent pharmacokinetic measures [42, 44, 83-85]. Maximal plasma concentrations are in general reached within one hour post-dose (i.e. at the end of infusion) and the elimination half-life is approximately 3-5 hours. In phase I studies, no changes in clearance,  $V_{ss}$  and  $t_{1/2}$  were observed across dose levels;  $C_{max}$  and  $AUC_{0-\infty}$  values increased proportionally over the entire range of tested doses, indicating that cilengitide exhibited linear pharmacokinetics. Mean volumes of distribution ranged from 33 to 56 L, and the mean clearance ranged from 5.72 to 9.62 L/hour. PK parameters after repeated dosing were similar to those after the first dose, indicating a lack of accumulation. There are no obvious differences in cilengitide pharmacokinetic parameters when administered per body surface area compared to flat dosing [42, 43]. No pharmacokinetic interaction was observed when cilengitide was co-administered with either TMZ [43] or CYP3A-enzyme inducing anti-epileptics [42].

Evaluation of 30 GBM tumor samples resected after prior treatment with cilengitide (doses administered preoperatively 8, 4 and 1 day before surgery, revealed that cilengitide reached the tumor tissue of GBM patients. There was a trend to higher exposure with higher dose; specifically cilengitide concentrations of 400 ng/gram of tumor were noted for patients treated at the 500 mg dose level and 1190 ng/gram for patients who received 2000 mg doses [86]. At the same time points, cilengitide was no longer detectable in the plasma in most patients, however, for patients with detectable plasma concentrations, the calculated tissue to plasma ratios were 1.83 for the 500 mg dose and 4.17 for the 2000 mg dose. The intra-tumor cilengitide concentrations measured 24 hours after last administration of the 2000 mg dose were in the concentration range for tumor inhibition predicted by pre-clinical models.

Data from individual adult GBM patients treated with 500 mg and 2000 mg cilengitide doses revealed CSF concentrations of approximately 1/100 of that achieved in plasma. Again, patients treated at the 2000 mg dose level had CSF levels in the range for tumor inhibition predicted by pre-clinical models. Maximum cilengitide CSF levels were obtained approximately 2 hours after those in the plasma, and the CSF profiles indicated a prolonged half-life of cilengitide in the CSF compared to plasma [9].

### **Pharmacodynamics and preclinical studies**

Cilengitide binds with 1:1 stoichiometry to  $\alpha v\beta 3$  with a  $K_D$  of 28nM (Cilengitide, Investigator's Brochure, Version 11.0). Cilengitide blocks binding of vitronectin to isolated  $\alpha v\beta 3$  and  $\alpha v\beta 5$  with an  $IC_{50}$  of 4 and 79 nM, respectively [87, 88]. Cilengitide inhibits vitronectin/integrin binding with an  $IC_{50}$  of 0.4  $\mu$ m in cell adhesion assays using M21 and UCLA-P3 human melanoma cell lines, respectively [87]. In contrast, cilengitide has no effect on cell adhesion mediated by the  $\alpha 1\beta 1$ ,  $\alpha 2\beta 1$ , or  $\alpha 5\beta 1$  integrins, nor does it affect fibrinogen binding to the platelet glycoprotein IIb/IIIa receptor, providing support for its target selectivity [87].

The anti-angiogenic activity of cilengitide has been demonstrated in the rabbit cornea retina and chicken chorioallantoic membrane models [88-90]. It also blocks proliferation and differentiation of human umbilical vein endothelial cells as well as human endothelial precursor cells [91, 92] and can induce endothelial cell apoptosis [93]. However, cyclic RGD-peptidomimetics can exert a biphasic effect on  $\alpha v \beta 3$  activity including antagonism at high concentrations and agonism at low concentrations [94]. Along these lines, a recent report demonstrated that use of an osmotic minipump to deliver sustained nanomolar concentrations of RGD-mimetic integrin inhibitors promoted angiogenesis and tumor growth in B16F0 melanoma and LLC lung carcinoma cells grown subcutaneously in syngeneic C57BL6 mice [95]. However, exposure to micromolar concentrations of integrin inhibitors, which are typically achieved following pulse dosing in ongoing clinical trials, inhibits angiogenesis as measured by either microvessel sprouting of mouse aortic rings incubated with VEGF or by quantification of tubule formation in a fibroblast-HUVEC co-culture model.

Cilengitide has demonstrated preclinical single-agent anti-tumor activity against melanoma [96] and orthotopic brain tumor xenografts [36, 71], which may be due to apoptosis induced by tumor cell detachment [71]. Recent preclinical work has focused on the clinical observation that MGMT methylated tumors tend to respond positively to cilengitide combined with XRT/TMZ compared to MGMT unmethylated tumors [43]. In these experiments, targeted alteration of MGMT expression in genetically engineered cell lines did not affect cilengitide activity [37], suggesting that the beneficial effect of cilengitide may be more related to its effect on the tumor microenvironment such as normalization of tumor vasculature to improve TMZ delivery or by exerting a stronger synergy with XRT/TMZ in chemosensitive tumors compared to chemotherapy-insensitive tumors. Finally, cilengitide has been shown to potentiate the anti-tumor activity of chemotherapy and radiation therapy in preclinical tumor models including GBM [37-40, 93, 97].

## **Clinical efficacy**

### Phase I studies

Table 2 highlights some of the clinical studies conducted to date with cilengitide in oncology; however, this review will focus primarily on studies targeting primary CNS tumors. The initial phase I trials of cilengitide prioritized the evaluation of safety and toxicity with secondary pharmacokinetic and correlative endpoints. An initial phase I study enrolled 37 patients with advanced solid tumors [85]. Cilengitide was administered intravenously over one hour twice weekly. In this study, no dose limiting toxicities (DLT) were observed and the MTD was not determined despite an escalation of cilengitide dosing in successive cohorts from 30 mg/m<sup>2</sup> up to 1600 mg/m<sup>2</sup>. Dose-dependent pharmacokinetics were observed and, as expected, increased in a dose proportional manner. Of note, the systemic exposure achieved at 120 mg/m<sup>2</sup> and above was within the concentration range for tumor inhibition predicted by pre-clinical models. An examination of serum angiogenic factors was also performed but failed to establish a pharmacodynamic correlation with stable or progressive disease. Three patients achieved stable disease but there were no objective radiographic responses.

Novel endpoints incorporating correlative assessments to determine biological activity were evaluated in a second phase I study in advanced solid tumor patients [83]. In this study cilengitide was again administered twice weekly as a 1-hour infusion beginning at a dose level of 120 mg/m<sup>2</sup>. Despite an escalation of the dose to 2400 mg/m<sup>2</sup>, no DLTs were observed and no MTD was identified. Endothelial cell apoptosis, gene expression profiles, systemic angiogenic factor measurements, and tumor tissue mean vessel density were assessed as potential biologic endpoints; however, none of these measures reliably predicted anti-tumor activity.

A phase I study of cilengitide limited to patients with recurrent malignant glioma was subsequently performed [42]. The rationale for a dedicated malignant glioma phase I study was the concern that CNS tumor patients may require a different MTD due to the risk of CNS hemorrhage or stroke potentially associated with anti-angiogenic or anti-vascular agents. A total of 51 patients enrolled on this study.

Cilengitide was escalated from 120 mg/m<sup>2</sup> to 2400 mg/m<sup>2</sup>. Infrequent and inconsistent toxicities were observed across administered dose levels and the MTD was not defined. Specific DLTs encountered included a grade 3 thrombosis, grade 4 myalgia/arthralgia, grade 3 anorexia/hypokalemia/hyponatremia, and grade 3 thrombocytopenia. There were no episodes of intracranial hemorrhage or stroke. Evidence of anti-tumor activity was observed both in higher and lower dose levels and included complete and partial responses in two and four patients, respectively, and stable disease for at least six months in six additional patients. Pharmacokinetic analyses from this study revealed that 1) the kinetics of cilengitide were linear, 2) flat dosing was feasible because dosing per body surface area did not affect drug clearance, and 3) concurrent use of either enzyme inducing anticonvulsants or corticosteroids did not affect cilengitide pharmacokinetics. Perfusion MR imaging and the measurement of plasma angiogenic growth factors were performed to identify potential biomarkers of anti-tumor activity. Decreased tumor perfusion was noted more commonly among patients treated with the higher cilengitide dose.

A companion dose escalation phase I study was performed in pediatric patients with recurrent malignant glioma and enrolled 31 patients. Intracranial hemorrhage occurred in 3 patients who were treated at the highest dose level (2400 mg/m<sup>2</sup>). However, two of these events were asymptomatic and it was unclear if these events were related to cilengitide or underlying tumor activity. One patient with a recurrent GBM experienced a complete response and two had stable disease. The recommended dose for subsequent studies in children is 1800 mg/m<sup>2</sup> [44].

### Phase II studies

A phase II study, with PFS-6 as the primary endpoint, was then performed in adults with GBM at first recurrence [9]. This study evaluated two dose levels of cilengitide including an intermediate-low (500 mg) dose and an intermediate-high (2000 mg) dose. The rationale for this design was based on results of the prior phase I study: 1) durable responses were observed across the spectrum of dose levels evaluated and 2) the MTD was not defined. Key eligibility criteria included the following: histologically confirmed

GBM that recurred following surgery, radiotherapy, and no more than one chemotherapy regimen; age  $\geq 18$  years; measurable ( $\geq 1 \text{ cm}^2$ ), contrast-enhancing tumor; KPS  $\geq 70$ ; and adequate bone marrow, hepatic and renal function. Patients were randomized to receive single-agent cilengitide at either 500 mg or 2000 mg per dose as a 1-hour infusion twice weekly with a 72 hour interval between infusions. Four-week treatment cycles were repeated until unacceptable toxicity, progressive disease (PD), or consent withdrawal. Treatment arms were stratified to equally enroll based on degree of pre-enrollment surgery (none vs. biopsy/subtotal resection) and KPS (70-80 vs. 90-100). The study enrolled 81 patients, including 41 on the 500 mg arm and 40 on the 2000 mg arm. Patients on both arms tolerated therapy well and there were no consistent significant toxicities observed overall, nor was there a difference in the incidence of adverse events between the two dose groups. Four patients experienced grade 3 non-hematologic toxicities that were possibly related to cilengitide therapy including single patients with transaminase elevation, arthralgia, weight gain and headache with altered mental status. There were no grade 4 or 5 study-related, non-hematologic events. Only one patient experienced an intracranial hemorrhage (grade 2) however this event occurred at the time of tumor progression. Pharmacokinetic evaluation revealed linear exposures for both plasma and cerebrospinal fluid. Although evidence of anti-tumor activity was observed for patients in both arms, those treated with the 2000 mg dose achieved higher rates of radiographic response (Figure 2), and improved PFS-6 and overall survival; however the randomized phase II study design was not powered to define superiority of one of the treatment arms (Table 3).

A parallel phase II study, conducted by the North American Brain Tumor Consortium (NABTC-0302), was designed to evaluate intratumoral penetration of cilengitide among recurrent GBM patients with no more than two prior episodes of tumor progression [86]. Patients were randomized to receive three doses of cilengitide at either 500 mg or 2000 mg per dose on days 8, 4 and 1 prior to planned surgical debulking. Cilengitide was continued after surgery at 2000 mg/dose twice weekly for all patients. Among the 30 patients enrolled, cilengitide was well tolerated. Eight patients developed grade 3/4



lymphopenia and one patient each experienced grade 3 fatigue, thrombocytopenia, myalgias and non-cardiac pulmonary edema. In addition, there were no episodes of peri-operative hemorrhage or wound healing difficulty. The average intratumoral concentration of cilengitide, assessed approximately 24 hours after the last cilengitide dose, were 400 and 1190 ng/gram of tissue for the 500 and 2000 mg dosing cohorts, respectively. The calculated tissue to plasma ratios were 1.83 for the 500 mg dose and 4.17 for the 2000 mg dose. Importantly, this study confirmed that cilengitide achieved effective GBM intratumoral penetration that was retained for at least 24 hours.

The cumulative clinical experience among recurrent GBM patients demonstrated that cilengitide as a single agent was well tolerated, effectively penetrated into GBM tumors and was associated with therapeutic benefit in some patients with recurrent GBM. Therefore, studies to evaluate cilengitide in newly diagnosed GBM were undertaken. Further support for this decision included preclinical orthotopic GBM xenograft data demonstrating that cilengitide enhanced the anti-tumor activity of radiation therapy in a schedule dependent manner [38]. Two parallel phase II clinical trials in newly diagnosed GBM were conducted. In the first study, 52 patients were enrolled in a single-arm, uncontrolled, multi-center study with PFS-6 as the primary endpoint [43]. All patients received cilengitide at 500 mg/dose twice weekly throughout standard XRT/TMZ followed by six cycles of adjuvant TMZ plus cilengitide. The protocol specified cilengitide administration continue until the end of 6 cycles of adjuvant TMZ; thereafter, patients were allowed to continue cilengitide on a voluntary basis, but only 7 patients elected to do so. Relevant patient characteristics included a median age of 57 years, an ECOG score of 0-1 in 92%, and a gross total resection in 44%. Thirty-three percent of patients were on CYP3A-inducing anti-epileptics (EIAEDs). MGMT gene promoter methylation status was available in 45 patients (87%) and 23 (51%) had evidence of MGMT methylation. Median follow-up of treated patients was 34 months. No significant toxicity was attributable to the addition of cilengitide to XRT/TMZ. Overall hematological toxicity was modest and occurred within the range expected for XRT/TMZ. Constitutive symptoms (nausea, headache,

fatigue, vomiting, and anorexia) were the most common non-hematologic AEs. Importantly, intracranial hemorrhage occurred rarely and within the range expected for XRT/TMZ without Cilengitide [3]. Fifty patients (96%) completed combination therapy with cilengitide plus concomitant XRT/TMZ, and 23 (44%) patients completed all six cycles of adjuvant TMZ plus cilengitide. Early discontinuation was due to progressive disease in 19 patients (37%), toxicity in nine patients (17%) and consent withdrawal in one patient (2%). Pharmacokinetic studies revealed that cilengitide exposure did not accumulate with repeated administration and that cilengitide metabolism was not affected by concurrently administered TMZ, XRT or EIAEDs. TMZ pharmacokinetics were concordant with previously published data,[98] indicating that cilengitide did not influence TMZ metabolism. With a median follow-up of 34 months, PFS-6 was 69% and median OS was 16.1 months. Patients with MGMT methylated tumors had significantly longer PFS ( $p < 0.001$ ) and OS ( $p = 0.022$ ) compared to those with unmethylated tumors (Table 4). Of note, PFS-6 and median OS were also higher for the MGMT methylated patients treated with cilengitide on this study compared to historical controls treated with standard therapy [47]. In contrast, patients on this study with unmethylated MGMT tumors had a similar outcome compared to those treated historically with standard therapy alone. The mechanism underlying a preferential benefit for cilengitide among newly diagnosed GBM patients with MGMT methylated tumors undergoing XRT/TMZ is not clear but could include 1) direct potentiation of the anti-tumor effect of TMZ and 2) an anti-angiogenic effect that normalizes tumor vasculature allowing greater TMZ delivery and/or oxygenation [99]. For both of these possible mechanisms, patients with MGMT methylated tumors would be predicted to achieve greater anti-tumor benefit from cilengitide compared to those with unmethylated tumors. In addition, a dose over 500 mg might be required to have an anti-tumor effect on GBM tumors with unmethylated MGMT status.

A second phase II study evaluating cilengitide for newly diagnosed GBM patients was conducted by the New Agents Brain Tumor Treatment (NABTT) cooperative group (NABTT 0306) [100]. In this study, cilengitide was administered twice weekly throughout standard XRT/TMZ, during the 6 monthly

cycles of adjuvant TMZ, and continued thereafter until PD. A safety lead-in period was included that enrolled three, six-patient cohorts who received cilengitide at 500 mg/dose, 1000 mg/dose and 2000 mg/dose, respectively. No DLTs were observed among any patients treated at these dose levels. Thereafter, 94 patients were enrolled to the phase II component of the study that included randomization to cilengitide doses of either 500 or 2000 mg. The median age of the 112 enrolled patients was 55 years, the median KPS was 90 and 76% of patients underwent a debulking craniotomy. Median overall survival for all patients was 18.9 months and overall survival at one year was 80%. Improved outcome was again observed with the 2000 mg dose level compared to the 500 mg dose. MGMT status was also assessed from archival tumor tissue using immunohistochemistry and a subgroup analysis of outcome based on MGMT status will be forthcoming.

Based on the encouraging results observed among recurrent and newly diagnosed GBM patients to date, a randomized phase III study (CENTRIC) evaluating the addition of cilengitide to standard XRT/TMZ and adjuvant TMZ followed by cilengitide maintenance compared to standard XRT/TMZ and adjuvant TMZ alone for newly diagnosed GBM patients with methylated MGMT tumors was initiated. Over 500 patients will be randomized with completed enrollment expected by early 2011 and preliminary outcome results by 2013. For patients with an unmethylated MGMT promoter, a companion phase II study of cilengitide (CORE) is evaluating safety, feasibility and efficacy of intensified, daily cilengitide (2000 mg up to five times per week) during XRT/TMZ chemoradiotherapy. Thereafter, all patients in this study will receive 6 cycles of standard 5-day TMZ cycles plus cilengitide dosed at 2000 mg twice weekly until progression, unacceptable toxicity, non-compliance or consent withdrawal. No DLTs were observed during the safety run-in component of this study. The phase II portion of the study is underway and randomizes patients to one of three arms (80 patients per arm) including: 1) cilengitide 2000 mg/dose daily for five days per week (Monday-Friday) with standard TMZ/XRT and then followed at 2000 mg twice weekly with adjuvant TMZ and subsequently as cilengitide maintenance, 2) cilengitide 2000 mg/dose twice weekly with standard TMZ/XRT and with adjuvant TMZ and subsequently as cilengitide

maintenance, and 3) standard TMZ/XRT and with adjuvant TMZ. A second randomized phase II study of cilengitide (CECIL) is evaluating the 1-year OS rate in newly diagnosed GBM patients treated with XRT/TMZ followed by 6 cycles of dose intensified TMZ (administered at a daily dose of 100 mg/m<sup>2</sup>/day for 21 out of 28 days) combined with 52 weeks of either cilengitide (at a dose of 2000mg twice a week) or cetuximab (at an initial dose of 400mg/m<sup>2</sup> followed by a once-weekly dose of 250 mg/m<sup>2</sup>) [www: ClinicalTrials.gov Identifier NCT01044225].

### **Safety and tolerability**

Cumulative experience with cilengitide to date indicates that it has a favorable safety profile. Phase I studies of cilengitide monotherapy among adult solid tumor and GBM patients did not identify dose dependent or dose-limiting toxicity despite wide dosing ranges of cilengitide tested (up to 2400 mg/m<sup>2</sup> which approximates 4000 mg twice weekly) [42, 44, 85]. Furthermore, no consistent toxicities have been observed in additional phase II studies [9, 43, 83, 85, 101, 102]. Among all adverse events reported, regardless of causality, among patients treated with cilengitide, the most common observed non-hematologic toxicities include fatigue, nausea, dyspnea, headache, peripheral edema, diarrhea, constipation and anorexia. Importantly, cilengitide is not associated with hemorrhages, delayed wound healing or wound dehiscence, or other adverse events when administered peri-operatively to GBM patients undergoing craniotomy [86]. In addition, cilengitide does not appear to exacerbate toxicity associated with chemotherapy or radiotherapy [43, 84].

### **Regulatory affairs**

Cilengitide is an investigational drug that is being evaluated in randomized, controlled clinical trials in GBM, non-small cell lung cancer and squamous cell carcinoma of the head and neck. Phase I/II trials are also ongoing for other indications (Table 3). An ongoing randomized, registration phase III study targets newly diagnosed GBM patients with MGMT methylated tumors. A complementary development program is active through the Cancer Therapy Evaluation Program (CTEP) of the US National Cancer Institute via

a Cooperative Research and Development Agreement (CRADA). Cilengitide has received orphan drug status for development in malignant glioma by both the European Medicines Agency and the United States Food and Drug Administration.

## **Conclusion**

Cilengitide is an RGD pentapeptidomimetic that blocks ligand binding and subsequent activation of the  $\alpha v\beta 3$  and  $\alpha v\beta 5$  integrins. Preclinical studies demonstrate anti-tumor activity in glioma and other solid tumors that can augment the activity of cytotoxic chemotherapy and radiotherapy. In addition, several preclinical assays indicate that cilengitide has anti-angiogenic activity. Clinical trials to date confirm a favorable safety profile and lack of MTD. Pharmacokinetic studies demonstrate predictable, linear pharmacokinetics and, in GBM patients, intratumoral and CSF concentrations above levels associated with anti-tumor activity in preclinical models. Durable radiographic responses, encouraging disease control and promising overall survival results, favoring the 2000 mg dose, have been achieved in a phase II study using cilengitide monotherapy among recurrent GBM patients. Two recently completed phase II studies also demonstrate encouraging overall survival results when cilengitide is combined with standard XRT/TMZ for newly diagnosed GBM patients. Accrual to an ongoing phase III trial in newly diagnosed GBM patients with a methylated MGMT promoter is nearing completion and this study will determine if the addition of cilengitide to standard XRT/TMZ improves overall survival compared to standard XRT/TMZ alone. Randomized clinical trials are also underway with cilengitide in non-small cell lung cancer and head and neck squamous cell carcinoma.

## **Executive summary**

### **Mechanism of action**

- Cilengitide is a cyclized RGD-containing pentapeptide (peptidomimetic) that blocks ligand binding and subsequent activation of the  $\alpha v\beta 3$  and  $\alpha v\beta 5$  integrins.

### **Pharmacokinetic properties**

- Cilengitide has a rapid distribution with maximum concentrations achieved within one hour. The mean elimination half-life is 3-5 hours. Concentrations above predicted preclinically active levels have been documented in tumors and cerebrospinal fluid of GBM patients 24 hrs after last cilengitide administration. Clearance is predominantly via the renal route.

### **Clinical efficacy**

- Durable radiographic responses, encouraging disease control, and promising overall survival results have been achieved in phase I and II studies using cilengitide monotherapy among recurrent GBM patients. Two recently completed phase II studies have also demonstrated encouraging overall survival when cilengitide was combined with standard XRT/TMZ for newly diagnosed GBM patients.

### **Safety and Tolerability**

- Side effects:
  - Several clinical trials confirm a favorable safety profile and lack of MTD as well as significant attributable toxicity. The most common observed non-hematologic adverse events in cilengitide trials include fatigue, nausea, dyspnea, headache, peripheral edema, diarrhea, constipation and anorexia.
- Precautions:
  - None
- Monitoring:
  - None
- Contraindications:
  - None other than documented hypersensitivity.
- Drug interactions:
  - None documented to date.
- Dosage and administration:
  - For adults, flat dosing of 2000 mg intravenously over one hour two days per week with doses separated by 72 hours (Monday and Thursday or Tuesday and Friday). Dosing among pediatric

patients has not been firmly established but the recommended dosing schedule for phase II studies is 1800 mg/m<sup>2</sup>.

### **Future perspective**

Integrins are attractive therapeutic targets for many malignancies given their myriad contribution to underlying tumor biology. To date, integrin targeting agents, including cilengitide, have been associated with a favorable safety profile. Cilengitide can be safely combined with chemotherapy and radiation therapy, and provides encouraging clinical anti-tumor activity so far. A recent preclinical study suggests that prolonged exposure to extremely low doses of RGD-mimetic integrin inhibitors may be associated with stimulation of tumor growth and angiogenesis [95]. Although this is not the dosing regimen used clinically with cilengitide and such findings have not been observed in the clinical application of anti-integrin agents to date[103] ongoing and future studies should evaluate such an adverse potential.

Overall, the ongoing randomized, controlled phase III (CENTRIC) and randomized, controlled phase II (CORE, ADVANTAGE, CERTO) studies will provide data on the clinical efficacy of cilengitide in GBM, NSCLC and SCCHN with clinically relevant doses and schedules.

A critical area of future focus will be the identification of biomarkers associated with durable benefit or early failure. Finally, the development of more convenient formulations for patients may facilitate improved compliance and overall patient satisfaction.

### **Information resources**

Merck Serono: [www.merckserono.com](http://www.merckserono.com)

### **Figure Legends**

**Figure 1.** Chemical structure of cilengitide.

**Figure 2.** Representative durable radiographic response of a patient with recurrent GBM treated with single-agent cilengitide.

**Financial & competing interests disclosure**

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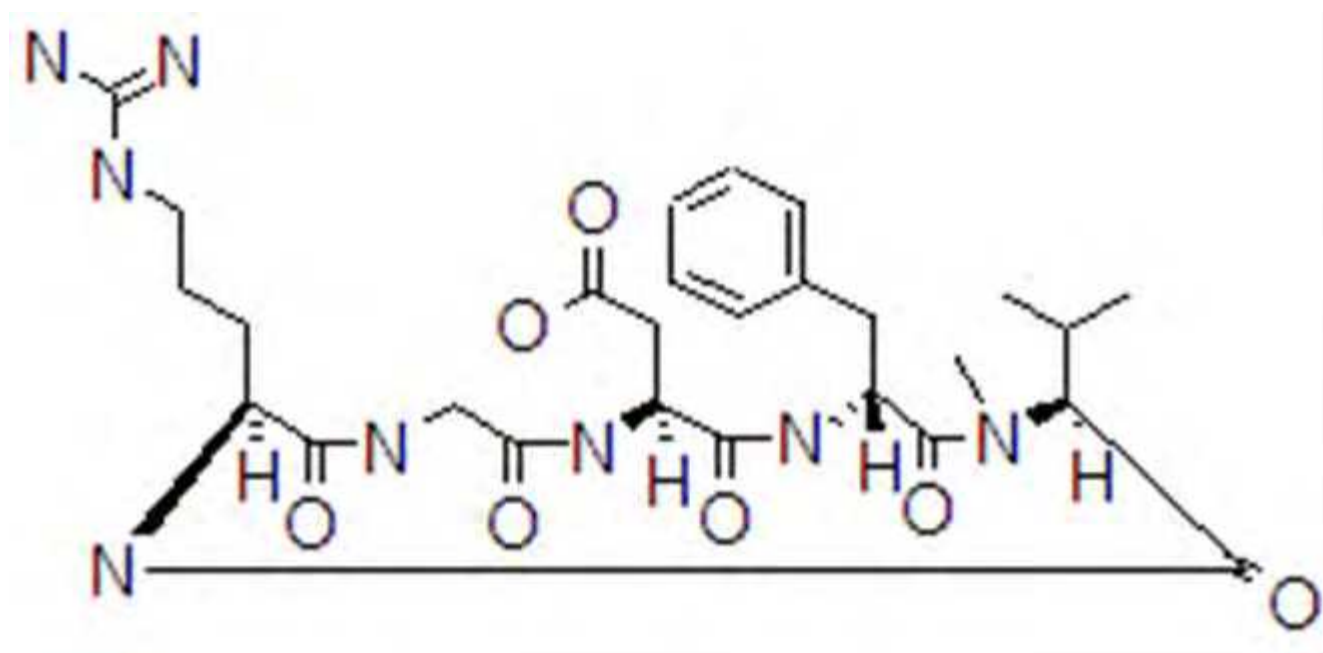


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Figure 1.



**Figure 2.** Representative radiographic response of a recurrent GBM patient treated with single agent cilengitide. This patient maintained this response for over 3.5 years.

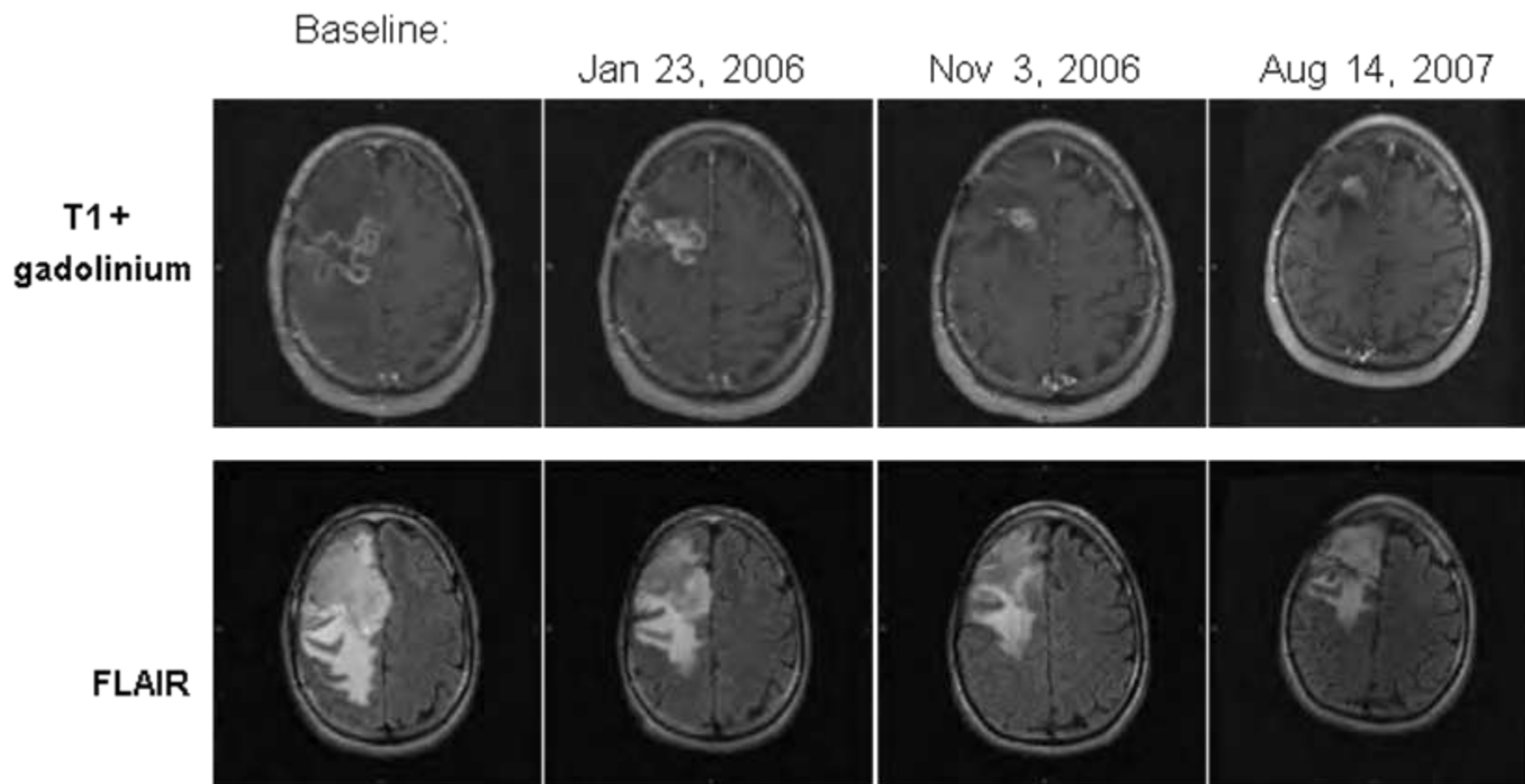


Table 1. Listing of integrin targeting agents in clinical development for oncology.

Agent	Company	Target	Class	Route of Administration	Indications	Phase of Development
Cilengitide	Merck Serono	$\alpha v\beta 3/5$	peptidomimetic	i.v.	Glioblastoma (SCCHN, NSCLC, other)	Phase III
intetumumab (CNT095)	Centocor	$\alpha v$	MAb	i.v.	HRPC (melanoma, solid tumours)	Phase II
E7820	Eisai	$\alpha 2$	Misc: Sulphonamide derivative	p.o.	CRC (lymphoma)	Phase II
Volociximab	Biogen	$\alpha 5\beta 1$	MAb	i.v.	RCC, melanoma (Pancreas)	Phase II
Etaracizumab (MEDI-522, Abegrin)	Medimmune	$\alpha v \beta 3$	MAb	i.v.	Prostate	Phase II
ATN 161	Attenuon	$\alpha 5\beta 1$	peptidomimetic	i.v.	Glioblastoma	Phase II
DI 17E6	Merck Serono	$\alpha v$	MAb	i.v.	CRC, Prostate	Phase II
MK0429	MSD	$\alpha v\beta 3$	Non-peptidomimetic	p.o.	HRPC	Phase I
GLPG 0187	Galapagos	5 integrin receptors	peptidomimetic	p.o./s.c.	Bone mets in mBC	Phase I
PF-04605412	Pfizer	$\alpha 5\beta 1$	MAb	i.v.	Solid Tumours	Phase I
IMGN388	ImmunoGen	$\alpha v$	MAb (DM4 cell-killing agent attached)	i.v.	Solid Tumours	Phase I
Celastrol	-	$\beta 1$ Integrin	Misc.	-	Prostate, Pancreas	Preclinical
HYD1	Moffitt Cancer Center	inhibitory peptide	peptidomimetic	-	Multiple myeloma	Preclinical
264RAD	AstraZeneca	Integrin $\alpha v\beta 6$ ligand	MAb	-	Oncology	Preclinical

Abbreviations: i.v. = intravenous; MAb = monoclonal antibody; Misc. = miscellaneous; p.o. = oral; s.c. = subcutaneous;

**Table 2.** Clinical trials with cilengitide in adults with malignant glioma (MG) and published or ongoing randomized controlled trials for other cancer indications.

Study	Study Population	Trial Phase	Trial Design	Dose (2X/week unless otherwise specified)	No. Patients	Reference
NABTT 9911	Recurrent MG	I	Dose escalation	200-2400 mg/m <sup>2</sup>	51	Nabors LB et al. 2007 <sup>42</sup>
PBTC-012	Refractory pediatric CNS tumors	I	Dose escalation	120-2400 mg/m <sup>2</sup>	33	MacDonald TJ et al. <sup>44</sup>
EMD 009	Recurrent GBM	II	Randomized	500mg – 2000 mg	81	Reardon DA et al. <sup>9</sup>
NABTC 0302	Recurrent GBM	II (peri-operative)	Randomized	500mg vs 2000 mg	30	Gilbert M et al. <sup>86</sup>
EMD 010	Newly diagnosed GBM	II	Single arm	500mg	52	Stupp R et al. <sup>43</sup>
NABTT 0306	Newly diagnosed GBM	II (with safety run-in)	Randomized	500mg vs 2000 mg	112	Grossman SA et al. <sup>100</sup>
CENTRIC	Newly diagnosed GBM; MGMT methylated	III	Randomized	2000 mg	504	Ongoing
CORE	Newly diagnosed GBM; MGMT unmethylated	II (with safety run-in)	Randomized	2000 mg 2-5X/week	240	Ongoing
CECIL	Newly diagnosed GBM; MGMT unmethylated	II	Randomized	2000 mg	108	Ongoing
NCI 3358	Advanced solid tumor	I/II	Single arm	600 mg/m <sup>2</sup> vs 1200 mg/m <sup>2</sup>	20	Harihan S et al. 2007 <sup>83</sup>
	Advanced solid tumor	I	Single arm	30 mg/m <sup>2</sup> -1600 mg/m <sup>2</sup>	37	Eskens FA et al. 2003 <sup>85</sup>
EMD 004	Pancreatic cancer	II	Randomized	600 mg/m <sup>2</sup>	89	Friess H et al. 2006 <sup>84</sup>
NCI 6735	Prostate cancer	II	Single arm	2000 mg	44	Bradley DA et al. 2010 <sup>101</sup>
NCI 6372	Prostate cancer	II	Randomized	500 mg vs 2000 mg	106	Ongoing
CERTO	Non-small cell lung cancer	II (with safety run-in)	Randomized	500-2000 mg	189	Ongoing
ADVANTAGE	Head and neck cancer	I/II	Randomized	500-2000 mg	194	Ongoing

Abbreviations: CNS – central nervous system; EMD: EMD Serono, Inc. (US affiliate of Merck KGaA, Darmstadt, Germany); GBM – glioblastoma; MG – malignant glioma; mg – milligrams; MGMT – methylguanine methyltransferase; NABTT – New Agents Brain Tumor Therapy Group; NABTC – North American Brain Tumor Consortium; NA – not available; NCI – National Cancer Institute

PBTC – Pediatric Brain Tumor Consortium

**Table 3.** Efficacy of single-agent cilengitide by dose level among recurrent GBM

Outcome or Response	Stratum A (n=41) (500 mg per day)	Stratum B (n=40) (2000 mg per day)
Number of patients with a radiographic response (%)	2 (5)	5 (13)
Median time to progression (weeks)	7.9	8.1
95% CI	7.7, 15.6	7.9, 15.0
6-month PFS (%)	10	15
95% CI	2.8, 23.7	5.7, 29.8
Overall survival (months)	6.5	9.9
95% CI	5.2, 9.3	6.4, 15.7
HR	0.70 [0.43, 1.14], p=0.15	
Number of patients completing ≥12 cycles (%)	3 (7)	5 (13)
Number of patients completing ≥24 cycles (%)	2 (5)	2 (5)



Table 4. Activity of cilengitide (500 mg/dose) among newly diagnosed GBM patients by MGMT methylation status compared with historical control.

	Cilengitide Phase II <sup>1</sup>			EORTC/NCIC <sup>2,3</sup>		
	All (n=52)*	MGMT methylated (n=23)	MGMT unmethylated (n=22)	RT + TMZ <sup>2</sup> (n=287)	MGMT methylated <sup>3</sup> (n=46)	MGMT unmethylated <sup>3</sup> (n=60)
Median						
PFS (months)	8 (6.0 – 10.7)	13.4 (8.6 – 22.8)	3.4 (2.3 – 7.1)	6.9 (5.8 – 8.2)	10.3 (6.5 – 14.0)	5.3 (5.0 – 7.6)
PFS – 6 (%)	69 (54 – 80)	91 (70 – 98)	41 (21 – 60)	53.9 (48.1 – 59.6)	68.9 (55.4 – 82.4)	40.0 (27.6 – 52.4)
Median						
Overall Survival (months)	16.1 (13.1 – 23.2)	23.2 (15.5 – NR)	13.1 (9.7 – 17.6)	14.6 (13.2 – 16.8)	21.7 (17.4 – 30.4)	12.7 (11.6 – 14.4)
Overall Survival						
12 months	68 (53 – 79)	91 (69 – 98)	51 (28 – 70)	61.1 (55.4 – 66.7)	NR	NR
24 months	35 (22 – 48)	46 (25 – 64)	20 (6 – 40)	26.5 (21.2 – 31.7)	46.0 (31.2 – 60.8)	13.8 (4.8 – 22.7)

\*data in parentheses is 95% confidence intervals unless otherwise indicated

<sup>1</sup>Stupp R et al. J Clin Oncol 28:2712-8, 2010

<sup>2</sup>Stupp R et al. N Engl J Med 352:987-96, 2005

<sup>3</sup>Hegi ME et al. N Engl J Med 352:997-1003, 2005